The Role of Leukotriene B\textsubscript{2} Receptor BLT1 in Experimental Lyme Arthritis


**Introduction**

- Leukotriene B\textsubscript{2} (LT\textsubscript{B}2) is a potent lipid chemotaxant derived from arachidonic acid (Figure 1). It is produced and released by neutrophils, mast cells, and macrophages during the innate immune response to infection.
- LT\textsubscript{B}2 binds to its high-affinity receptor BLT1, located predominantly on the surface of leukocytes.
- Upon binding, LT\textsubscript{B}2 induces neutrophil and monocyte chemotaxis to the site of inflammation.
- LT\textsubscript{B}2 and BLT1 act in concert to produce tissue inflammation during autoimmune arthritis.
- Previous studies in autoimmune arthritis have shown that inhibition of BLT1 prevents recruitment of neutrophils and macrophages.
- We hypothesized that C3H BLT\textsuperscript{1-/-} mice infected with *Borrelia burgdorferi* would exhibit less inflammation than C3H wild-type (WT) mice, as well as a decreased ability to clear *Borrelia*.

**Materials and Methods**

**Ear Tissue Borrelia Loads:**

- Stock cultures of virulent, N40 strain of *Borrelia burgdorferi* were grown to 10\textsuperscript{8} bacteria/mL. WT C3H/HeJ and BLT1 KO mice were inoculated with 10\textsuperscript{7} bacteria intraperitoneally.
- Animals were sacrificed 21 and 42 days post-infection.

**Ankle Swelling Curve:**

- Ankle swelling was measured on days 21 and 35 post-infection. Ankle swelling measurements were then determined by subtracting the initial baseline measurement from subsequent measurements.

**Ankle Histology:**

- Eicosanoid levels were determined by HPLC analysis using a Lipoxin A4 Enzyme Immunoassay Kit from Ogden Biomedical Research, respectively.
- Stock cultures of mouse *Borrelia burgdorferi* were grown at 35\textdegree C in BSK medium to 1x10\textsuperscript{8} bacteria/ml. WT C3H wild-type (WT) and BLT1 KO mice were inoculated with 10\textsuperscript{7} bacteria intraperitoneally.
- Animals were sacrificed 21 and 42 days post-infection.

**Results**

- Arthritis severity scores demonstrate that the development of Lyme arthritis at the time of peak inflammation on day 21 appeared delayed in BLT1 KO as compared to C3H WT mice. However, 35 days post-infection, the arthritis severity scores of BLT1 KO mice were higher than WT mice, though not statistically significant. *Borrelia* ear loads were similar between both strains of mice. Flow cytometry data revealed similar cell populations between both mouse strains.

**Future Directions**

- Repeat experiment to confirm current findings.
- Submit ankle tissue samples to more accurately measure levels of LT\textsubscript{B}2, Lipoxin A\textsubscript{4}, and other eicosanoids using MS/MS analysis.
- Examine tissue samples on day 60 post-infection to definitively conclude whether there is a delay in resolution of arthritis.
- Measure levels of inflammatory and anti-inflammatory cytokines such as IL-12, IL-10, IL-6, IL-1\beta, and TNF-\alpha.
- Repeat experiment evaluating BLT2, the low-affinity LT\textsubscript{B}2 receptor, in C3H BLT\textsuperscript{2-/-} or inhibited mice.

**Conclusions**

1. *Borrelia* loads were not statistically different between the two strains, suggesting that another mechanism is at work which is clearing the pathogen in the absence of BLT1-mediated neutrophil and macrophage recruitment.
2. We expected LT\textsubscript{B}2 levels to be increased in BLT1 KO mice, as the eicosanoid would be unable to bind to its receptor, but was not statistically different between the two strains of mice.
3. There appears to be a delay in development of arthritis in BLT1 KO mice.

**Figure 1:** Simplified schematic of eicosanoid production, emphasizing leukotriene synthesis and their receptors.

**Figure 2:** (A) LT\textsubscript{B}2 enhances macrophage phagocytosis. (B) Lack of LT\textsubscript{B}2 delays resolution.

**Figure 3:** BLT1 KO mice exhibited similar swelling as C3H WT mice on consecutive days post-infection.

**Figure 4:** BLT1 KO mice exhibited similar swelling as C3H WT mice on consecutive days post-infection.

**Figure 5:** Multiplex qR-PCR. Borrelia clearance from ear tissue was similar between mouse strains.

**Figure 6:** Eicosanoid levels in C3H WT and BLT1 KO mice, measured on days 21 and 35 post-infection.

**Figure 7:** Cell counts between BLT1 KO and C3H WT mice as measured on days 21 and 42 were not statistically different.

**Figure 8:** Histology severity scores demonstrate that the development of Lyme arthritis at the time of peak inflammation on day 21 appeared delayed in BLT1 KO as compared to C3H WT mice. However, 35 days post-infection, the arthritis severity scores of BLT1 KO mice were higher than WT mice, though not statistically significant. *Borrelia* ear loads were similar between both strains of mice. Flow cytometry data revealed similar cell populations between both mouse strains.